

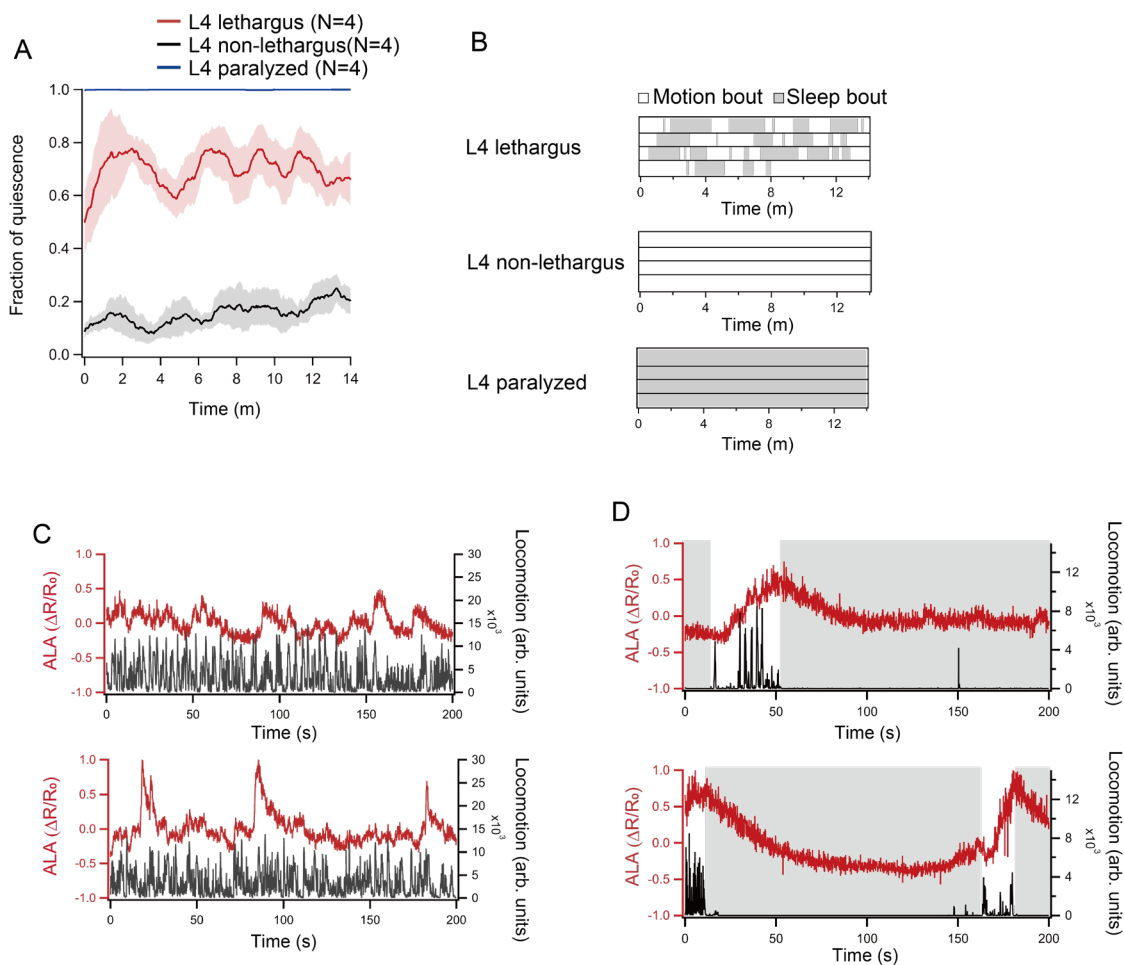
iScience, Volume 25

Supplemental information

**Intracellular Ca^{2+} dynamics in the ALA neuron
reflect sleep pressure and regulate
sleep in *Caenorhabditis elegans***

Shinichi Miyazaki, Taizo Kawano, Masashi Yanagisawa, and Yu Hayashi

1 **SUPPLEMENTAL INFORMATION**



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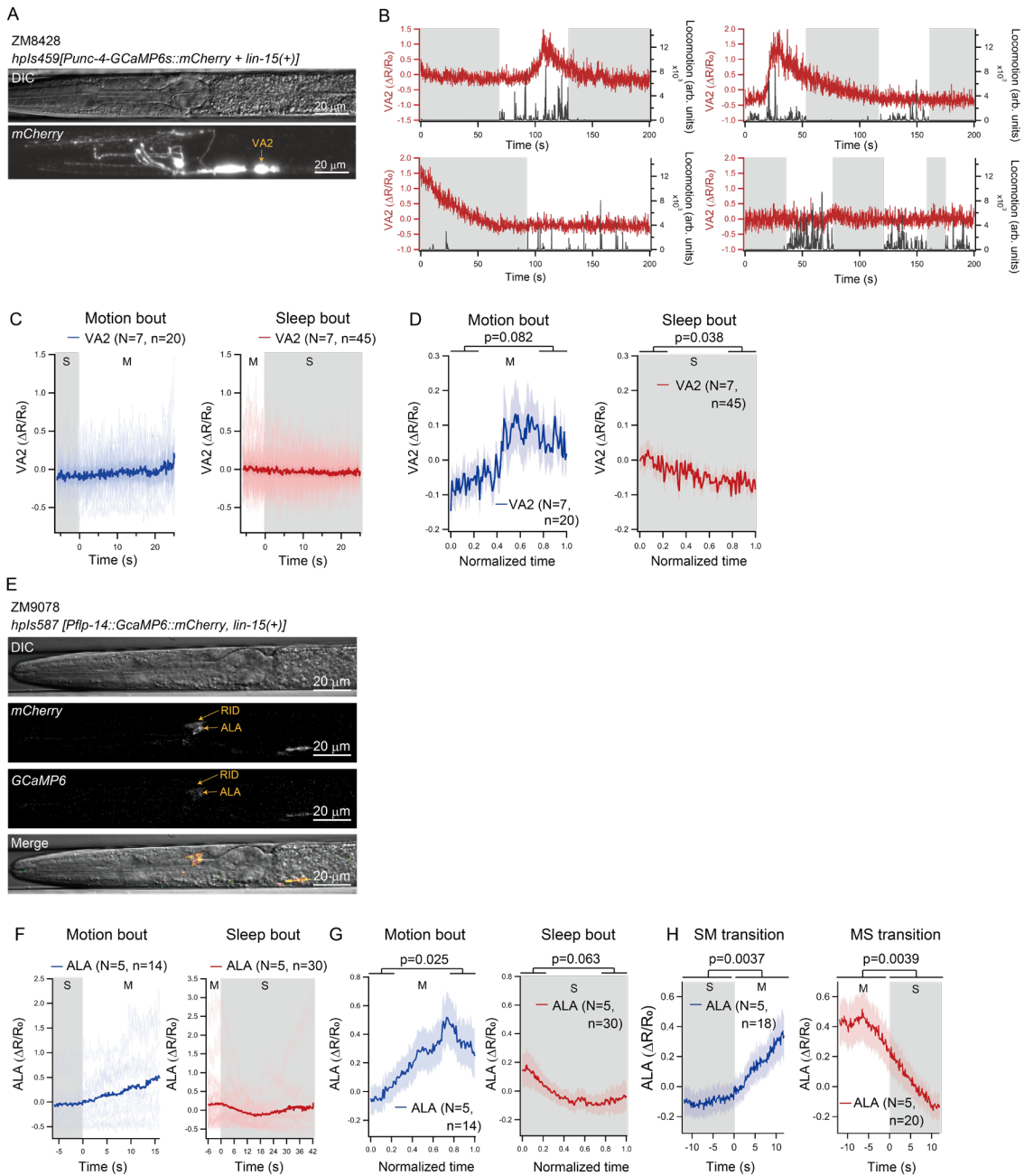
3 **Figure S1.** Detailed analyses of the behavior and ALA intracellular Ca^{2+} dynamics in AML70 *lite-1(ce314);*
 4 *wtfls5[Prab-3::NLS::GcaMP6s; Prab-3::NLS::tagRFP]* (related to Figures 1 and 2)

5 (A, B) Comparison of the fraction of quiescence (A) and the representative patterns of sleep bouts and motion
 6 bouts (B) among L4 lethargus, non-lethargus, and paralyzed larvae. Shaded areas in (A) indicate \pm SEM . N in
 7 the figure represent numbers of animals.

8 (C) Representative patterns of ALA Ca^{2+} activity (red) and locomotor activity (black) in L4 non-lethargus
 9 larvae.

10 (D) Additional representative patterns of ALA Ca^{2+} activity (red) and locomotor activity (black) in L4
 11 lethargus larvae. Grey areas represent sleep bouts.

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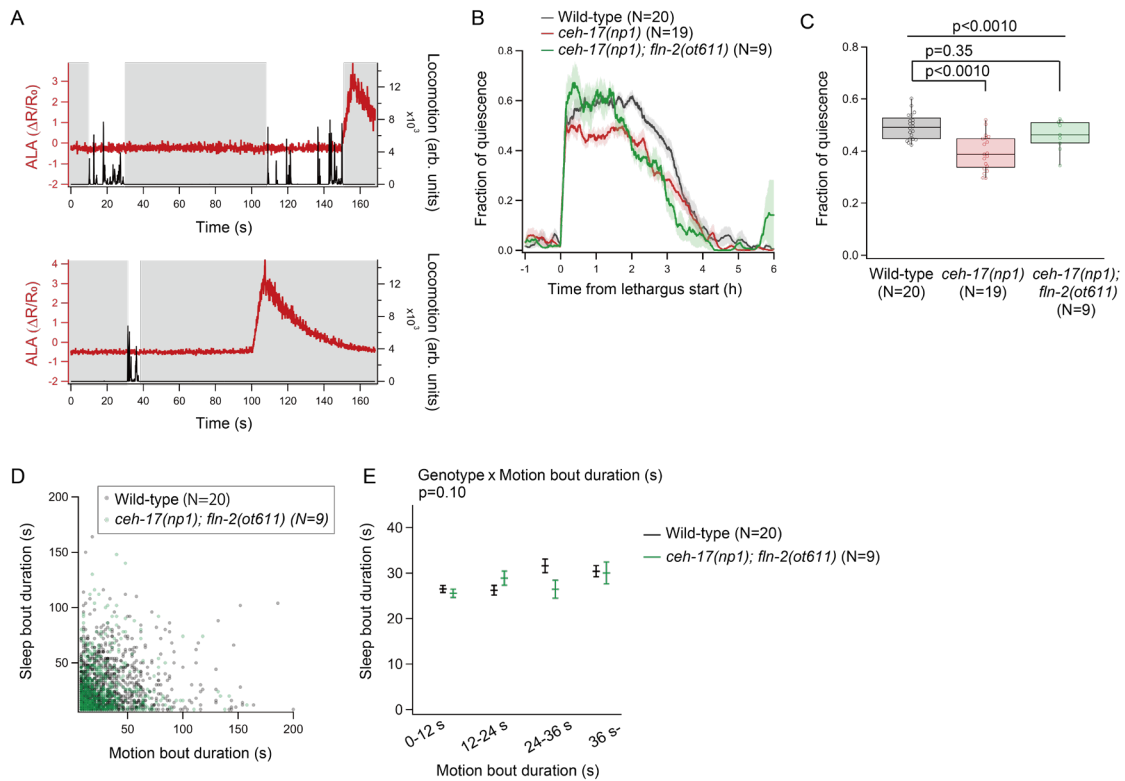
14 **Figure S2.** Ca²⁺ imaging of the VA2 and ALA (related to Figures 2 and 3)

15 (A) Expression pattern of *mCherry* in ZM8428 *hpls459 [unc-4p::GCaMP3::mCherry + lin-15(+)]*.

16 (B) Representative patterns of VA2 intracellular Ca²⁺ activity (red) and locomotor activity (black). Grey areas
 17 represent sleep bouts.

18 (C) Individual (light blue or red) and averaged (dark blue or red) dynamics of VA2 intracellular Ca²⁺ during

19 motion bouts (left) or sleep bouts (right). Grey areas represent sleep bouts.
20 (D) Averaged dynamics of intracellular Ca^{2+} and locomotor activity along motion bouts (left) or sleep bouts
21 (right). Here, the duration of each sleep or motion bout was normalized to 1. Shaded areas indicate \pm SEM. P-
22 values in paired t-test (motion bout) and Wilcoxon signed-rank test (sleep bout) are indicated.
23 (E) Expression patterns of *mCherry* (middle) and *GCaMP6* (lower) in ZM9078 *hpIs587 [Pflp-*
24 *14::GcaMP6::mCherry, lin-15(+)]*.
25 (F) Individual (light blue or red) and averaged (dark blue or red) dynamics of ALA intracellular Ca^{2+} during
26 motion bouts (left) or sleep bouts (right) in ZM9078 *hpIs587 [Pflp-14::GcaMP6::mCherry, lin-15(+)]*. Grey
27 areas represent sleep bouts.
28 (G) Averaged dynamics of intracellular Ca^{2+} and locomotor activity along motion bouts (left) or sleep bouts
29 (right). Here, the duration of each sleep or motion bout was normalized to 1. Shaded areas indicate \pm SEM. P-
30 values in paired t-test (motion bout) and Wilcoxon signed-rank test (sleep bout) are indicated.
31 (H) Averaged dynamics of Ca^{2+} activity around SM and MS transitions. Shaded areas indicate \pm SEM. P-
32 values in paired t-test are indicated. Grey areas represent sleep bouts.
33 N and n in the figure represent numbers of animals and bouts, respectively.
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35

36 **Figure S3.** Detailed analyses of strains carrying *ceh-17(np1)* (related to Figure 3)

37 (A) Additional representative patterns of Ca^{2+} activity (red) and locomotor activity (black) in the *ceh-17(np1)*
 38 mutant (SLP930 *ceh-17(np1); lite-1(ce314); wtfIs5[Prab-3::NLS::GcaMP6s + Prab-3::NLS::tagRFP]*).

39 Grey areas represent sleep bouts.

40 (B) Averaged patterns of fraction of quiescence around lethargus in wild-type (black), SLP769 *ceh-17(np1)*
 41 mutant (red), and IB16 *ceh-17(np1); fln-2(ot611)* mutant (green). The same data as in Figure 3C are shown
 42 for wild-type and SLP769. Shaded areas indicate \pm SEM.

43 (C) Comparison of the mean fraction of quiescence among wild-type, SLP769 *ceh-17(np1)*, and IB16 *ceh-*
 44 *17(np1); fln-2(ot611)*. The same data as in Figure 3D are shown for wild-type and SLP769. P-values in the
 45 Tukey multiple comparison test are indicated.

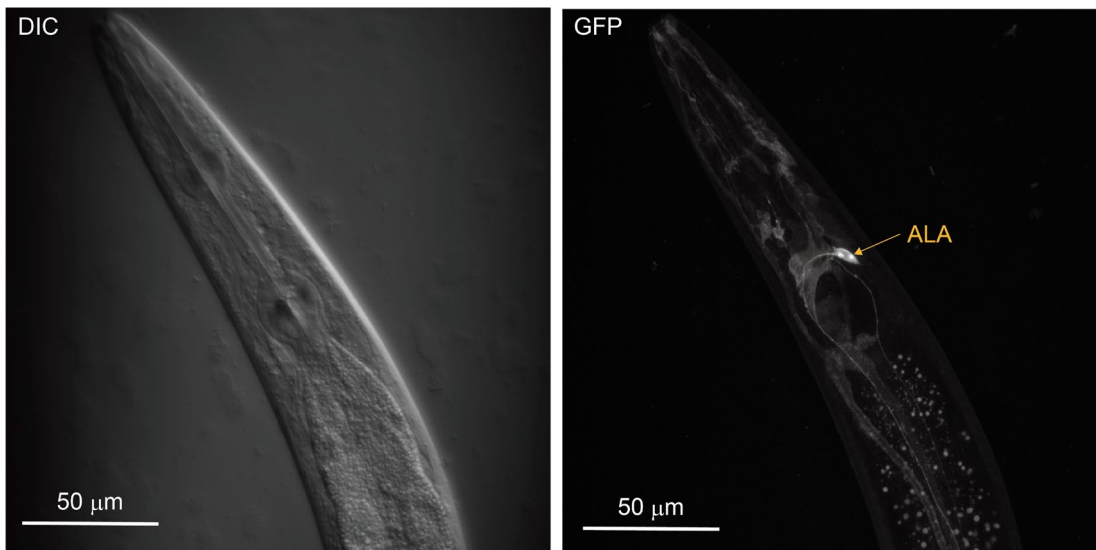
46 (D) Sleep bout duration plotted against the prior motion bout duration in wild-type (black) and IB16 *ceh-*
 47 *17(np1); fln-2(ot611)* mutants (green). The same data as in Figure 3I are shown for wild-type.

48 (E) Comparisons of the distribution of sleep bout durations based on the duration of the prior motion bouts in
 49 wild-type (black) and IB16 *ceh-17(np1); fln-2(ot611)* mutants (green). The same data as in Figure 3J are
 50 shown for wild-type. P-value in the interaction between genotype and motion bout duration in two-way
 51 ANOVA is indicated above the graph.

52 N and n in the figure represent numbers of animals and bouts, respectively.

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SLP983 *remEx394*[*Pver-3::ChR2(C128S)::GFP*]



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55 **Figure S4.** Expression pattern of ChR2(C128S)::GFP in SLP983 *remEx394*[*Pver-3::ChR2(C128S)::GFP*]
56 (related to Figure 4)

57 Representative confocal image of SLP983 *remEx394*[*Pver-3::ChR2(C128S)::GFP*].

58 DIC image (left) and fluorescent image (right) are shown. Arrow indicates the ALA cell body.

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